Claims

1. A method of modulating differentiation, adhesion and/or survival of a cell presenting the neural cell adhesion molecule (NCAM), said method comprising

a) providing a compound capable of interacting with the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules of NCAM by

i) interacting with the Ig1 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig1 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

ii) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig1 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iii) interacting with the Ig2 module of NCAM, and thereby mimicking the interaction between Ig2 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iv) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

v) interacting with the Ig2 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig2 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules,

b) providing an NCAM presenting cell;

c) modulating the differentiation, adhesion and/or survival of the at least one NCAM presenting cell by contacting the NCAM presenting cell of (b) with the compound of (a).

2. The method of claim 1, wherein the differentiation, adhesion and survival are mediated by NCAM.

3. The method of claims 1-2, wherein the NCAM is mammalian NCAM, or a variant, or a fragment thereof.

4. The method of claim 3, wherein the NCAM has the sequence identified as SwissProt Ass. No: P13591 (SEQ ID NO: 44), or a variant, or a fragment thereof.

5. The method of claim 1, wherein the compound is selected from the group comprising peptides, carbohydrates, lipids, and co-polymers of amino acids with other organic molecules.

6. The method of claim 5, wherein the peptides are peptide fragments derived from the sequence of NCAM identified as SwissProt Ass. No: NP_113709 (SEQ ID NO: 44) or SwissProt Ass. No: P13591 (SEQ ID NO: 45), or variants of said peptide fragments.

7. A method for testing a compound whether it is capable of modulating interaction between two individual NCAM molecules through a homophylic binding site composed of the Ig1, Ig2 and Ig3 modules of said NCAM molecules by modulating the interaction of

i) the Ig1 module of one individual NCAM molecule with the Ig3 module of another individual NCAM molecule, and/or

ii) the Ig2 module of one individual NCAM molecule with the Ig3 module of another individual NCAM molecule, and/or

iii) the Ig2 module of one individual NCAM molecule with the Ig2 module of another individual NCAM molecule

said method comprising

a) providing a compound;

b) providing at least one individual fragment of an NCAM molecule, wherein said fragment comprising a sequence of consecutive amino acid residues corresponding to the sequence of the Ig1-2-3 module of NCAM comprising residues 1 to 289 of the sequence set forth in SEQ ID NO: 44, or a fragment of said individual fragment;

c) testing whether the compound is capable of

i) interacting with the Ig1 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig1 and Ig3 modules of NCAM, wherein said modules are from the two individual fragments of (b) interacting to each other, and/or

ii) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig1 modules of NCAM, wherein said modules are from the two individual fragments of (b) interacting to each other, and/or

iii) interacting with the Ig2 module of NCAM, and thereby mimicking the interaction between Ig2 and Ig3 modules of NCAM, wherein said modules are from the two individual fragments of (b) interacting to each other, and/or

iv) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig2 modules of NCAM, wherein said modules are from the two individual fragments of (b) interacting to each other, and/or

v) interacting with the Ig2 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig2 and Ig2 modules of NCAM, wherein said modules are from the two individual fragments of (b) interacting to each other.

by contacting the compound with a fragment of (b);

e) selecting a compound capable of at least one interaction of (c) as a candidate compound capable of modulating differentiation, adhesion and/or survival of a cell presenting NCAM.

8. The method of claim 7, wherein the candidate compound is selected from the group comprising peptides, carbohydrates, lipids and co-polymers of amino acids with other organic molecules.

9. The method of claim 8, wherein the peptides are peptide fragments derived from the sequence of NCAM having SwissProt Ass. No: NP_113709 (SEQ ID NO: 44) or the sequence of NCAM having SwissProt Ass. No: P13591 (SEQ ID NO: 45), or fragments or variants of said peptide fragments.

10. The method of claim 7, wherein the individual fragment of NCAM, or a fragment thereof, is a soluble protein.

11. A crystal of a polypeptide comprising the Ig1-2-3 module of NCAM comprising at least 289 consecutive amino acid residues from the sequence of rat NCAM having SwissProt Ass. No: NP_113709 (SEQ ID NO: 44).

12. The crystal according to claim 11, wherein the polypeptide comprises aa 1 to 289 of SEQ ID NO: 44.

13. The crystal according to claim 11, wherein the polypeptide consists of aa 1 to 289 of SEQ ID NO: 44 and an extra amino acid sequence of 1 to 4 amino acids residues.

14. The crystal according to claim 11, wherein said crystal diffracts X-rays for determination of atomic co-ordinates to a resolution of at least 4 Å.

15. The crystal according to claim 11, wherein the crystal effectively diffracts X-rays for the determination of the atomic coordinates to a resolution at most 5. 0 Å.

16. The crystal according to claims 14 or 15, wherein the crystal effectively diffracts X-rays for the determination of the atomic coordinates to a resolution 1. 5 Å.

17. The crystal according to claim 11, wherein said crystal comprises atoms arranged in a spatial relationship represented by the structure co-ordinates of Table 2 (Figure 2) or by coordinates having a root mean square deviation therefrom of not more than 2.5 Å.

18. The crystal according to claim 11, wherein said crystal has unit cell dimensions of a=51.5 Å, b=108.5 Å, c= 149.0 Å, alpha=90°, beta=90°, gamma=90°.

19. A method of preparing a crystal as defined in claims 11-18, said method comprising the steps of

i) providing said polypeptide;

ii) optionally providing a compound capable of interacting with said polypeptide;

iii) growing the crystal under conditions wherein said polypeptide, and optionally said compound, is incubated in a buffer comprising in the range of 5 to 25% polyethylene glycol, in the range of 0.01 M to 0.5M salt, in the range of 1 to 10% of an alcohol selected from the group consisting of glycerol and 2-methyl-2,4-penthanediol, wherein said buffer has a pH in the range of 6 to 9;

iv) thereby preparing said crystal.

20. A method for selecting a candidate compound capable of modulating differentiation, adhesion and/or survival of NCAM presenting cells by modulating the interaction of

i) the Ig1 module of one individual NCAM molecule with the Ig3 module of another individual NCAM molecule, and/or

ii) the Ig2 module of one individual NCAM molecule with the Ig3 module of another individual NCAM molecule, and/or

iii) the Ig2 module of one individual NCAM molecule with the Ig2 module of another individual NCAM molecule,

said method comprising the steps of

a) providing a soluble or a crystalline polypeptide comprising the Ig1-2-3 module of NCAM,

b) generating a structural model of the Ig1-2-3 module of NCAM of (a) by using the computer modelling techniques;

c) in-silico evaluating compounds for the capability of

i) interacting with the Ig1 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig1 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

ii) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig1 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iii) interacting with the Ig2 module of NCAM, and thereby mimicking the interaction between Ig2 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iv) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

v) interacting with the Ig2 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig2 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules,

by using the structural model(s) of the Ig1-2-3 module of NCAM of (b);

d) selecting a candidate compound capable of at least one interaction of (c), and

e) testing the candidate compound of (d) in an in vitro or in vivo assay for the capability of modulating differentiation, adhesion

and/or survival of NCAM presenting cells, said assays comprising at least one NCAM presenting cell, and /or

f) testing the candidate compound of (d) in an assay comprising evaluating the capability of the compound of at least one interaction of (b) by contacting the compound with at least one individual fragment of an NCAM molecule, said fragment comprising a sequence of consecutive amino acid residues corresponding to the sequence of the Ig1-2-3 module of NCAM comprising residues 1 to 289 of the sequence set forth in SEQ ID NO: 44.

21. The screening method of claim 20, wherein the computer generated model is the structural model of a crystalline protein according to any of the claims 13-20.

22. The screening method of claim 20, wherein the computer generated model is the structural model of the Ig1-2-3 module in solution.

23. A compound capable of interacting with an NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules of NCAM by

i) interacting with the Ig1 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig1 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

ii) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig1 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iii) interacting with the Ig2 module of NCAM, and thereby mimicking the interaction between Ig2 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iv) interacting with the Ig3 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

v) interacting with the Ig2 module of NCAM, and thereby mimicking and/or modulating the interaction between the Ig2 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules.

24. The compound of claim 23, wherein the compound is selected from the group consisting of
WFSPNGEKLSPNQ (SEQ ID NO: 1),
YKCVVTAEDGTQSE (SEQ ID NO: 2),
TLVADADGFPEP (SEQ ID NO: 3),
QIRGIKKTD (SEQ ID NO: 4),
DVR (SEQ ID NO: 5),
RGIKKTD (SEQ ID NO: 6),
DVRRGIKKTD (SEQ ID NO: 7),
KEGED (SEQ ID NO: 8),
IRGIKKTD (SEQ ID NO: 9),
KEGEDGIRGIKKTD (SEQ ID NO: 10),
DKNDE (SEQ ID NO: 11),
TVQARNSIVNAT (SEQ ID NO: 12),
SIHLKVFAK (SEQ ID NO: 13),
LSNNYLQIR (SEQ ID NO: 14),
RFIVLSNNYLQI (SEQ ID NO: 15),
KKDVRFIVLSNNYLQI (SEQ ID NO: 16),
QEFKEGEDAVIV (SEQ ID NO: 17),
KEGEDAVIVCD (SEQ ID NO: 18),
GEISVGESKFFL (SEQ ID NO: 19),
KHIFSDDSSELTIRNVDKNDE (SEQ ID NO: 20),
AFSPNGEKLSPNQ (SEQ ID NO: 40),
AKSVVTAEDGTQSE (SEQ ID NO: 41),
DVRRGIKKTD (SEQ ID NO: 42),
QIRGIKKTD (SEQ ID NO: 43).

25. The compound according to claim 23 or 24, wherein the compound is a candidate compound selected by the method according to claim 7 or claim 20.

26. The compound according to claim 25, said compound having the amino acid sequence WFSPNGEKLSPNQ set forth in SEQ ID NO: 1, fragments or variants thereof.

27. The compound according to claim 25, said compound having the amino acid sequence YKCVVTAEDGTQSE set forth in SEQ ID NO: 2, fragments or variants thereof.

28. The compound according to claim 25, said compound having the amino acid sequence TLVADADGFPEP set forth in SEQ ID NO: 3, fragments or variants thereof.

29. The compound according to claim 25, said compound having the amino acid sequence QIRGIKKTD set forth in SEQ ID NO: 4, fragments or variants thereof.

30. The compound according to claim 25, said compound having the amino acid sequence DVR set forth in SEQ ID NO: 5, fragments or variants thereof.

31. The compound according to claim 25, said compound having the amino acid sequence RGIKKTD set forth in SEQ ID NO: 6, fragments or variants thereof.

32. The compound according to claim 25, said compound having the amino acid sequence DVRRGIKKTD set forth in SEQ ID NO: 7, fragments or variants thereof.

33. The compound according to claim 25, said compound having the amino acid sequence KEGED set forth in SEQ ID NO: 8, fragments or variants thereof.

34. The compound according to claim 25, said compound having the amino acid sequence IRGIKKTD set forth in SEQ ID NO: 9, fragments or variants thereof.

35. The compound according to claim 25, said compound having the amino acid sequence KEGEDGIRGIKKTD set forth in SEQ ID NO: 10, fragments or variants thereof.

36. The compound according to claim 25, said compound having the amino acid sequence DKNDE (SEQ ID NO: 11), fragments or variants thereof.

37. The compound according to claim 25, said compound having the amino acid sequence TVQARNSIVNAT (SEQ ID NO: 12), fragments or variants thereof.

38. The compound according to claim 25, said compound having the amino acid sequence SIHLKVFAK (SEQ ID NO: 13), fragments or variants thereof.

39. The compound according to claim 25, said compound having the amino acid sequence LSNNYLQIR (SEQ ID NO: 14), fragments or variants thereof.

40. The compound according to claim 25, said compound having the amino acid sequence RFIVLSNNYLQI (SEQ ID NO: 15), fragments or variants thereof.

41. The compound according to claim 25, said compound having the amino acid sequence KKDVRFIVLSNNYLQI (SEQ ID NO: 16), fragments or variants thereof.

42. The compound according to claim 25, said compound having the amino acid sequence QEFKEGEDAVIV (SEQ ID NO: 17), fragments or variants thereof.

43. The compound according to claim 25, said compound having the amino acid sequence KEGEDAVIVCD (SEQ ID NO: 18), fragments or variants thereof.

The compound according to claim 25, said compound having the amino acid sequence GEISVGESKFFL (SEQ ID NO: 19), fragments or variants thereof.

44. The compound according to claim 25, said compound having the amino acid sequence KHIFSDDSSELTIRNVDKNDE (SEQ ID NO: 20), fragments or variants thereof.

45. The compound according to claim 25, said compound having the amino acid sequence AFSPNGEKLSPNQ (SEQ ID NO: 40), fragments or variants thereof.

46. The compound according to claim 25, said compound having the amino acid sequence AKSVVTAEDGTQSE (SEQ ID NO: 41), fragments or variants thereof.

47. The compound according to claim 25, said compound having the amino acid sequence DVRRGIKKTD (SEQ ID NO: 42), fragments or variants thereof.

48. The compound according to claim 25, said compound having the amino acid sequence QIRGIKKTD (SEQ ID NO: 43), fragments or variants thereof.

49. Use a compound obtainable of the method of claim 7 or 20 for modulating differentiation, adhesion and/or survival of NCAM presenting cells.

50. Use of one or more compounds as defined in any of the claims 23-48 for the manufacture of a medicament.

51. The use of claim 50, wherein the medicament is for treating normal, degenerated or damaged NCAM presenting cells.

52. The use of claim 50, wherein the medicament is for treatment comprising the stimulation of differentiation and/or survival of NCAM presenting cells.

53. The use of claim 50, wherein the medicament is for treating the diseases and conditions of the central and peripheral nervous system, or of the muscles or of various organs.

54. The use of claim 50, wherein the medicament is for treating the diseases or conditions of the central and peripheral nervous system, such as postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression; for treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplantation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of

the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel.

55. The use of claim 50, wherein the medicament is for treating the postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression.

56. The use of claim 50, wherein the medicament is for promoting the wound-healing.

57. The use of claim 50, wherein the medicament is for treating the cancer.

58. The use of claim 50, wherein the medicament is for preventing the cell death of heart muscle cells, such as after acute myocardial infarction, or after angiogenesis.

59. The use of claim 50, wherein the medicament is for promoting the revascularsation.

60. The use of claim 50, wherein the medicament is for stimulating the ability to learn and/or of the short and/or long-term memory.

61. Use of a crystal of the Ig1-2-3 module of NCAM for the in-silico screening a candidate compound capable of modulating NCAM homophylic adhesion-dependent neural plasticity, cell differentiation and/or survival.

62. A pharmaceutical composition comprising one or more compounds as defined in any of the claims 23-48.